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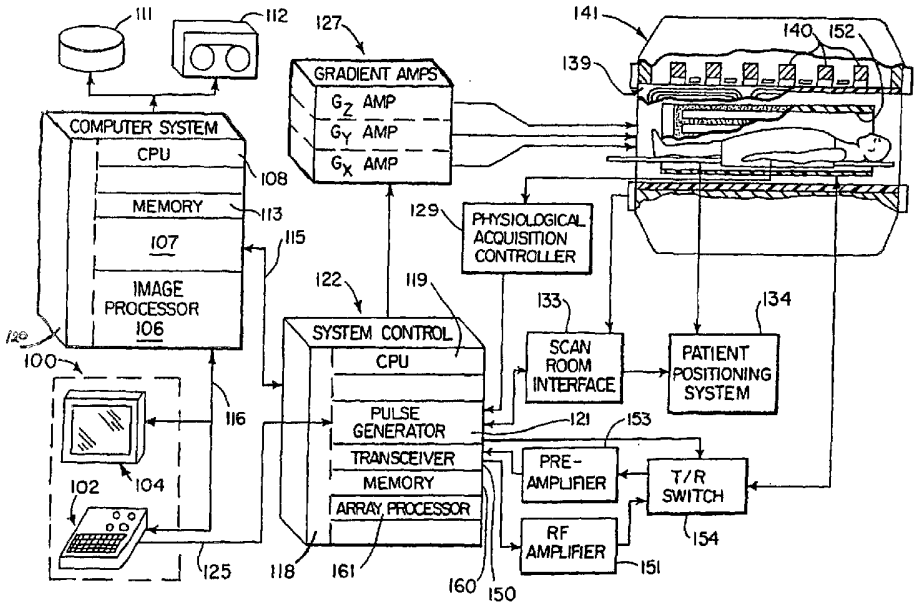

(57) Abstract: Polymeric contrast agents are used to test for blood vessel permeability, such as, for example, to diagnose sepsis. The agent is injected and images are obtained at various time intervals after injection. For increased accuracy, the blood circulation levels of the agents may optionally be monitored.

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METHOD FOR ASSESSING CAPILLARY PERMEABILITY

BACKGROUND

TECHNICAL FIELD

This disclosure relates to methods for assessing capillary permeability using contrast agents and imaging techniques, such as, for example, magnetic resonance imaging (MRI).

BACKGROUND OF RELATED ART

Sepsis is a major cause of morbidity and mortality in humans and other animals. Sepsis has become the leading cause of death in intensive care units among patients with non-traumatic illnesses. It is also the leading cause of death in young livestock (e.g., neonatal calves), and is a common medical problem in neonatal foals.

Sepsis can result from septicemia (i.e., organisms, their metabolic end-products or toxins in the blood stream), including bacteremia (i.e., bacteria in the blood), as well as toxemia (i.e., toxins in the blood), including endotoxemia (i.e., endotoxin in the blood).

Sepsis essentially comprises clinical evidence of bacterial infection and symptoms characterized by (i) temperatures in excess of about 101° F., or less than 96° F., (ii) heart rates greater than 90 beats per minute, and (iii) respiratory rates in excess of about 20 breaths per minute. Such symptoms are often followed by hypothermia, nausea, vomiting and diarrhea. Sepsis syndrome comprises sepsis together with evidence of altered organ perfusion. Septic shock essentially comprises sepsis syndrome together with hypotension. Septic shock symptoms include tachycardia, hypotension, disseminated intravascular coagulation, and the leakage of plasma proteins into the tissues (vascular leakage syndrome). Vascular leakage syndrome, essentially the leakage of plasma proteins from blood vessels into the surrounding tissues, is related to the occurrence of the following symptoms: peripheral cyanosis,

oliguria, edema and end-organ failure which may occur during the advanced stages of sepsis and may result in the death of the patient. Death typically results from renal, respiratory or cardiac failure or a combination thereof.

The onset of late stage sepsis is accompanied by release of tumor necrosis factor which acts on the capillaries and renders them leaky. However, the tests for the TNF factor are complicated by time dependent pulse emission of the factor into the blood stream. Many patients with sepsis or septic shock exhibit a rapid decline over a 24-48 hour period. Rapid methods of diagnosis and treatment delivery are essential for effective patient care.

Therefore, it would be advantageous to have a rapid method for diagnosing sepsis or septic shock to correctly identify this condition so there is little to no delay in the initiation of a suitable course of treatment.

SUMMARY

Polymeric contrast agents are used in accordance with this disclosure to test for capillary permeability. The agent is injected intravenously and an MR signal at a pre-selected region of interest (such as, for example, muscle) is recorded at various time intervals after injection. For increased accuracy, the blood circulation levels of the agents may optionally be monitored by imaging a major blood vessel such as the vena cava.

Suitable polymeric contrast agents include a paramagnetic entity complexed with a substituted polymeric carrier molecule. The polymeric carrier molecule has a polymer backbone substituted with a steric hindrance molecule. The steric hindrance molecule can be any molecule that by its physical size enforces an elongated conformation by providing steric hindrance between neighboring steric hindrance molecules. Preferably the steric hindrance molecule is neutral in charge or presents negative charges in an aqueous environment along the polymer chain to assist in keeping the polymer backbone straight through coulombic repulsion. The preferred substituted polymeric carriers have a length 5 to 500 times greater than their diameter, a net negative charge, and form a worm-like chain conformation with a long persistence

length. In particularly useful embodiments, lanthanide complexes (e.g., gadolinium-diethylenetriamine pentaacetic acid complexes) are attached to the polymer backbone to create complex molecules which are introduced into a blood vessel of the subject.

These complex molecules do not pass through normal capillary walls. Once the vascular structure becomes leaky, the penetration of the defective capillary wall by the complex molecules is enhanced by the worm-like configuration of the complex molecule which allows the molecule to "snake" around fixed obstacles and pass through the capillary wall.

BRIEF DESCRIPTION OF THE DRAWINGS

While the novel features of the invention are set forth with particularity in the appended claims, the invention, both as to organization and content, will be better understood and appreciated, along with other objects and features thereof, from the following detailed description taken in conjunction with the drawing, in which:

FIG. 1 is a reaction scheme for preparing highly conjugated polymers in accordance with one embodiment of this disclosure.

FIG. 2 is an illustration of the functioning of the present polymeric contrast agents in a subject.

FIG. 3 is an illustration of inter-strand and intra-strand cross-linking of polypeptides.

FIG. 4 an illustration of a highly substituted polypeptide useful as the polymeric contrast agent in the present methods.

FIG. 5 is a block diagram of an MRI system useful in the performance of the methods described herein.

FIG. 6 is a graphic representation of a pulse sequence performed by the MRI system of FIG. 5 to practice an embodiment of the methods described herein.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Methods for sensitive measurements of changes in blood capillary permeability are accomplished in accordance with this disclosure by administering a polymeric contrast agent to a subject having or suspected of having sepsis, septic shock or other condition resulting in vascular leakage syndrome. The use of polymeric contrast agents and imaging techniques allows for rapid imaging and hence rapid diagnosis of vascular leakage syndrome.

Suitable polymeric contrast agents include a paramagnetic entity complexed with a substituted polypeptide carrier molecule. The polymeric contrast agents have a length that is 5 to 500 times greater than their diameter, a net negative charge, and form a worm-like chain conformation with a long persistence length The worm-like configuration of the complex molecule is achieved by attaching a sufficient number of steric hindrance molecules along the polypeptide chain to eliminate or reduce intra-chain ionic bonds as well to allow charge repulsion between chelating moieties to unfold and extend the polymer chain. The amount of substitutions (also referred to as the degree of conjugation) thus affects the configuration of the resulting complex, with a higher degree of conjugation providing a more consistent extended structure and better diagnosis. A degree of conjugation of above 90% is typically required for the proper polymer configuration to be realized in the case of a carrier molecule having a lysine homopolymer backbone. Lower degrees of conjugation can be tolerated for certain carrier molecules having an amino acid copolymer backbone, such as, for example, a backbone that is a copolymer containing lysine and either glutamic or aspartic acid.

The present carrier molecules include a polymer backbone that is substituted with steric hindrance molecules which facilitate the attachment of a paramagnetic entity and which, due to their physical size, provide a physical restraint on polymer bending.

The nature of the polymer backbone is not critical, provided that the polymer has pendant groups which can be reacted with an activated SHM as described below to provide a polymer-SHM copolymer having an elongated structure. Suitable pendant groups which may be present ion the polymer include, but are not limited to amine groups, carboxyl groups and hydroxyl groups. Useful polymers include homo- and

co-polymers of poly(amino acids), poly(vinyl amine), poly(4-aminostyrene), poly(acrylic acid), poly(methacrylic acid), poly(carboxynorbornene), and dextran. Preferably, the polymer is a polypeptide. The polypeptide can be an amino acid homopolymer or a copolymer of two or more amino acids. Preferably, the polypeptide is selected from the group consisting of polylysine, polyglutamic acid, polyaspartic acid, copolymers of lysine and either glutamic acid or aspartic acid. Other polymers may be used provided that after reaction with the SHM, the resulting copolymer has an elongated structure characterized by a molecular length that is 5 to 500 times the cross-sectional diameter of the copolymer molecule and a net negative charge in an aqueous environment. In addition, the polymer preferably is of sufficient length to increase the time in which the product circulates in the blood. For polypeptides, the polymer backbone can advantageously be from 35 to 1500 amino acid residues long. Because the polymeric backbone is synthetic, the length can be tailored to provide desired residence times in the body. Clearance from the blood is rapid for short molecules, resulting in a short plasma lifetime. Plasma lifetime increases rapidly as the polymers increase in length. For example, where the polymer is a polypeptide, a plateau is reached for a molecular length of about 500 residues and little further change in lifetime occurs. Not only does the use of a synthetic polypeptide provide the ability to modify the polymer length so as to change the blood circulation times to smaller values, but the ability to modify the polymer length to probe small permeability modulations is also provided.

A PREFERRED HOMOPLOYMER IS A LYSINE HOMOPOLYMER

Where a copolymer forms the backbone of the carrier molecule, the coploylmer preferably contains lysine units and either glutamic acid units, aspartic acid units, or both. Glutamic and/or aspartic acid units may constitute from about 20 to about 60 percent of the copolymer. Preferably, the copolymer is a glutamic acid-lysine copolymer. Particularly useful copolymers have glu:lys ratios of about 1:4 for long (>400 residue) polymer constructs and ratios of about 6:4 for short (<200 residue) polymer constructs. A high content of lysine is believed advantageous for imaging as it allows a high loading of the copolymer with paramagnetic ions. Without wishing to be bound by any theory, it is believed that the presence of glutamic acid residues in

the copolymer backbone accomplishes two things. First, it is believed that the glutamic acid residues provide a stiffer initial copolymer backbone for the synthesis of the complete construct. Second, it is believed that the presence of glutamic acid residues in the copolymer promotes extension of the final polymer through charge repulsion.

At least a portion of the polymer backbone have steric hindrance molecules substituted thereon. The steric hindrance molecule ("SHM") can be any molecule that by its physical size enforces a elongated conformation by providing steric hindrance between neighboring steric hindrance molecules. Preferably the SHM is neutral in charge or presents negative charges in an aqueous environment along the polymer chain to assist in keeping the polymer backbone straight through coulombic repulsion.

In particularly useful embodiments, the SHM contains or chelates an imaging producing entity. Suitable imaging producing entities include paramagnetic entities, entities which undergo nuclear reaction resulting in release of detectable radiation. Non-limiting examples include ions which release alpha particles, gamma particles, beta particles, or positrons. Such image producing entities are known to those skilled in the art. Gamma emitters include, for example, In-^{111} In and Gd-^{153}. Positron emitters include, for example, Zr-^{89}, which may be employed in positron emission tomography (PET) imaging.

Particularly preferred steric hindrance molecules are molecules that chelate with paramagnetic entities. As those skilled in the art will appreciate, paramagnetic entities include certain transition metals and lanthanide ions. Any molecule known to complex with paramagnetic entities and which is of sufficient size to provide steric hindrance against polymer bending can be used as the SHM. Preferably, the SHM has a net negative charge. Suitable lanthanide ion chelating molecules include, but are not limited to diethylenetriaminepentaacetic acid (DTPA), 1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), 1,4,7,10-Tetraazacyclododecane-1,4,7,10- tetrakis(2-propionic acid) (DOTMA), 1,4,8,11-Tetraazacyclotetradecane-1,4,8,11-tetraacetic acid (TETA), 1,4,7,10-Tetraazacyclododecane -1,4,7,10-tetrakis[3-(4-carboxyl)-butanoic acid], 1,4,7,10-

Tetraazacyclododecane-1,4,7,10-tetrakis(acetic acid-methyl amide), 1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetrakis(methylene phosphonic acid), and p-Isothiocyanatobenzyl-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (p-SCN-Bz-DOTA).Ligands useful for chelating for other ions (such as, for example, Fe(III), Mn(II), Cu(II), etc.) include Bis(thiosemicarbazone) and derivatives, Porphyrins and derivatives, 2,3-Bis(2-thioacetamido)propionates and derivatives, *N,N'*-bis(mercaptoacetyl)-2,3-diaminopropanoate, and Bis(aminoethanethiol) and derivatives.

To attach the SHM to the polymer, an activating group is provided on the SHM. The activating group present on the SHM can be any group which will react with a polymer. Suitable groups include, but are not limited to mixed carbonate carbonic anhydride groups, succinimidyl groups, amine groups and dicyclohexylcarbodiimide (DCC) groups. Those skilled in the art will readily envision reaction schemes for providing an activating group on any given SHM.

In one embodiment, the SHM is DTPA and the activating groups are mixed carbonate carbonic anhydride groups. In particularly useful embodiments, a substantially mono-activated SHM is provided. The term "activated" means that a functional group is present on the molecule which permits covalent bonding of the molecule to appropriate amino acids. By the term "substantially mono-activated" it is meant that about 90% or more of the steric hindrance molecules contain only a single activated site. Mono-activation is believed to more consistently result in high levels of conjugation. A typical reaction scheme for activating DTPA and reacting it with a polypeptide backbone is shown in Fig. 1. As seen therein, a monoanhydride-DTPA is first prepared. Specifically, a flask is charged with acetonitrile and DTPA. Triethylamine is then added via syringe. The solution is warmed to 60°C. under a nitrogen atmosphere. The mixture is stirred until homogeneous. The clear solution is then cooled to -45°C. under nitrogen atmosphere and isobutyl chloroformate is slowly added to result in the mono-anhydride of DTPA. As those skilled in the art will appreciate, DTPA has five acid groups available for conversion to anhydride. However, since substantially mono-activated DTPA is desired, only one of these acid sites should be converted to anhydride. It has unexpectedly been found that the slow

addition of the chloroformate while cooling below -40°C. accomplishes this result, i.e., that about 90% or more of the DTPA is a monoanhydride of DTPA.

The activated SHM is then reacted with the polymer backbone. The precise conditions for reacting the polymer with the activated SHM will depend upon a number of factors including the particular polymer chosen and the specific SHM used. Those skilled in the art will readily envision reaction schemes for any given pair of materials to produce the desired substituted polymer product.

In a particularly useful embodiment, for example, the monoanhydride-DTPA described above is simply added dropwise to an aqueous solution of polylysine under ambient atmospheric conditions.

In another example, where the reactive pendant groups on the polymer backbone are electrophilic groups (such as, for example, a carboxylic acid groups), the anhydride of DTPA described above can be reacted overnight with a diamine (in which the diamine is in large excess to the anhydride). Ethylene diamine is a suitable choice, giving in the end a DTPA linkage of the desired length to achieve proper steric hindrance against polymer chain bending. The product is separated from the diamine and from DTPA which was not reacted, by ion exchange chromatography. The product is substantially mono-amine DTPA. Where the substantially mono-activated steric hindrance molecule is the foregoing monoamine-DTPA, it can be linked to a carboxyl group contining polymer (such as, for example, poly-glutamic acid) by a carboxyl coupling method. The carboxy acid groups of the polymer are activated by a coupling reagent, such as, for example, 1 Ethyl-3-(3-Dimethylaminopropyl) carbodiimide Hydrochloride (EDC) (Pierce, Rockford, Ill.). The activated carboxy acid groups on the polymer are then combined with the monoamine-DTPA to produce an amide linkage of the DTPA to the polymer backbone as a sidechain which acts as a steric hindrance straightening the polymer backbone.

The resulting polymer-steric hindrance molecule copolymer is then purified. During purification, the polymer-SHM copolymer is separated from the volatile solvents and other impurities. Any known techniques can be used to purify the polymer-SHM copolymers.

In a particularly useful embodiment, where a polypeptide backbone is used, a purification scheme is employed which does not result in complete drying of the polymer-SHM copolymer. It has unexpectedly been determined that excessive dryness affects the configuration of the copolymer and interferes with the determination of degree of conjugation.

A preferred purification scheme involves first exposing the reaction mixture to reduced pressure to remove impurities that are more volatile than water. Care should be taken not to remove all water from the reaction mixture during this step. The next step in this preferred purification scheme is to centrifuge the remaining reaction mixture. Soluble impurities remain in the supernatant fluid. The retentate from the centrifuge step is resuspended and subjected to dialysis. Optionally, ultrafiltration is performed on the dialyzed polymer. Techniques for these processes are within the purview of those skilled in the art.

The resulting product can then be characterized using any technique known to those skilled in the art, such as, for example, high performance liquid chromatography (HPLC).

Once the polymer-SHM copolymer is obtained, an image producing entity is incorporated into the conjugated polymer. Thus, for example, to achieve a MR active agent, a paramagnetic ion (such as, for example, gadolinium) can be incorporated into the product polymer chelating DTPA groups by dropwise addition of $GdCl_3$ in 0.1 M HCl (50 mM in Gd) into the polymer solution (15 mM $NaHCO_3$). The dropwise addition of Gd continues until a slight indication of free Gd (not chelated by available DTPA groups) is noted (small aliquots of polymer solution added to 10 microMolar of arzenzo IIII in acetate buffer--free Gd turns the dye solution blue).

In certain embodiments, the conjugated polymer can also be used for delivery of a therapeutic agent. It is also contemplated that a therapeutic agent can be attached at a few sites along the substituted polymer chain. By way of example, chemotherapeutic agents (such as, for example, doxorubicin) which have been shown to have activity against tumors can be attached to the conjugated polymer. For therapy, one could attach also use a radiotherapieutic agent (such as, for example ^{90}Y, or ^{211}At at a few

sites along the substituted polymer chain. The therapeutic entity can be attached to the conjugated polymer using techniques known to those skilled in the art. It is also contemplated that, the polymer backbone can be highly conjugated with a non-therapeutic SHM which chelates an imaging agent and a therapeutic agent can be bound to the SHM at a few sites along the substituted polymer chain, rather than being bound directly to the polymer backbone.

In certain embodiments, the conjugated polymer can also have a targeting moiety attached thereto. It is contemplated that a targeting moiety can be attached at a few sites along the substituted polymer chain. The targeting moiety can be attached to the conjugated polymer using techniques known to those skilled in the art. It is also contemplated that targeting moieties can be bound to the SHM at a few sites along the substituted polymer chain, rather than being bound directly to the polymer backbone.

In FIG. 2, capillary 10 is schematically shown having portions 11a, 11b with normal capillary structure and a diseased, leaky portion 11c. Pores are generally thought to be intercellular junctions which separate to cause leakiness in the capillaries. Normally, pores 15 of capillary 10 are small and polymeric contrast agent molecules 14 (being a polypeptide carrier molecule with an attached paramagnetic entity) are contained within capillary 10. In areas affected by sepsis or other condition leading to vascular leakage syndrome, pores 17 develop which are much larger than that of pores 15 in the normal portion of the capillary. Polymeric contrast agent molecule 14a is shown passing through pore 17a, out of capillary 10. Molecules 14b of polymeric contrast agent have already passed through diseased portion 11c of capillary 10 and have accumulated at a relatively higher concentration in tissue adjacent thereto compared to the concentration of the agent within the healthy portions 11a, 11b of capillary 10.

In the event that the pores are not simple channels, a process called reptation allows elongated worm-like molecules to wiggle around obstacles, and to pass through restricted openings, that globular or coiled molecules would be unable to pass through.

The present polymeric contrast agent molecules have a cross sectional diameter which is larger than that of the pores of normal capillaries such that they are contained within

the blood vessels in the normal state but have a cross sectional diameter smaller than that of the pores of the vessels in leaky capillaries such that they may readily pass out of the capillary and into the surrounding tissue. Polymeric contrast agents having a diameter of approximate 20-50 Angstroms (Å) generally pass through pore structures of the leaky vessels, but not that of normal capillaries.

In order to be effective at concentrating outside of a leaky blood vessel, the polymeric contrast agent molecules also advantageously can have a length long enough to increase the time in which they circulate in the blood, but small enough to pass out of the vessel. Once outside the vessel, longer molecules tend to remain there. In addition, very large macromolecular agents may not provide enough signal due to the changes in capillary permeability, while the small agents presently in clinical use penetrate normal capillaries to begin with so that changes would be more difficult to detect.

An elongated, worm-like conformation of a macromolecule results in greater uptake than other conformations, such as folded, or globular conformations. Conformation may be measured by a persistence length of the molecule. This may be determined by light scattering.

Conformation is a result of intra-chain charge interaction, and rigidity of the molecule. In certain embodiments, the polymeric backbone of the carrier molecules are selected to be polypeptides. However, many polypeptides tend to fold into tight random coils due to the relatively free rotation around each peptide bond. Also, if each polypeptide is composed of opposite charge amino acids, then intra-chain charge interaction as shown by bond 21 in FIG. 3. Inter-chain charge interaction between chains may also occur as shown by bond 23 of FIG. 3. If there is significant intra-chain charge interactions, the polymer-SHM copolymer may assume a globular, or folded, conformation.

The conformation attained by the present polymeric contrast agents is that of a worm-like shape being essentially a stretched out, extended chain with little folding. A measure of the "straightness" of a molecule is a persistence length. Persistence length is related to a radius of gyration, measured by light scattering experiments. A folded

polypeptide such as poly-L-lysine (PLL) with little or no substitution, has a low persistence length of about 10 Angstroms (Å), and is not suitable for diagnosing sepsis. Therefore, the present polymeric contrast agents preferably have a persistence lengths of 100-600 Å.

It is sometimes difficult to measure the persistence length of certain molecules by light scattering to determine their conformation because of the effects of contaminant particles in the test solutions. However, it was found that by measuring the magnetic resonance (MR) T_1 relaxation of a paramagnetic entity attached to the carrier, one could infer the conformation of the molecules of interest. This is performed by attaching paramagnetic ions, such as gadolinium, to the chelators along the polymer chain

When the carrier molecule is in an elongated conformation, the chelator/MR active entity is free to rotate about its attachment point to the main chain, allowing a long T_1 relaxation time of the surrounding water protons which are the source of the MR signal.

When the carrier molecule is in a globular or highly folded conformation, steric hindrance, and molecular crowding causes interaction with the chelator/MR active entity restricting rotation about its bond to the main chain. Thus, the chelator/MR active entity moves only with the general slow motion of the carrier molecule. This produces a short T_1 relaxation time.

A high relaxivity is associated with a molecule which folds upon itself into a globular conformation, such as albumen, at about 15 $sec.^{-1}$ $milliMolar^{-1}$ ($sec.^{-1}$ mM^{-1}). A low relaxivity is associated with an elongated molecule such as highly substituted Gd-DTPA PLL^h in which the Gd can rotate rapidly, having a relaxivity of about 8 $sec.^{-1}$ mM^{-1}. The optimum conformation of the present invention is associated with a relaxivity of 7-8 $sec.^{-1}$ mM^{-1}. When the relaxivity of a peptide agent was high, the uptake coefficient of such an agent was invariably low, evidently due to the absence of the reptation mechanism.

Since many in-vivo chemical entities have a negative charge, molecules introduced into the subject can advantageously have a net negative charge to reduce agglutination and to allow for stable long circulation in the blood plasma. It is known that negatively charged dextran molecules undergo glomerular filtration at a much slower rate than equivalent dextran molecules of positive charge or neutral charge.

The high net negative charge is also desirable since it also assists in the polymer-SHM copolymer retaining its elongated, worm-like conformation.

In FIG. 4 a polymeric contrast agent having a plurality of side chains substituting the hydrogen atoms is shown. The polymeric contrast agent is comprised of a plurality of amino acids 31, each linked end to end through a polypeptide bond. A plurality of side residues 33 are attached which cause steric hindrances and repulsion to straighten the copolymer chain.

FIG. 4 also shows that the length of the polymeric contrast agent should be significantly longer than its diameter by approximately 5 to 500 times. This causes the polymeric contrast agent and any attached chemical entities to pass through diseased capillary walls as discussed above.

It may be that in some applications, long blood circulation times would be undesirable. The present methods/materials provide the ability to reliably make short polymers of the desired worm-like conformation which allows the possible tailoring of blood circulation time to certain target levels. Blood circulation time is directly dependent on polymer chain length. The signed response is fast and the clearance from the blood circulation is rapid for shorter polymer lengths, both of which may be desirable in certain clinical screening procedures.

The polymeric contrast agent molecules used in accordance with certain embodiments of the present disclosure do not normally accumulate in other organs such as muscle, kidney or liver. Therefore, the present agents are particularly well suited for imaging of leaky blood vessels compared to over other imaging agents that are based on globular proteins or coiled polymers, which tend to show accumulation in liver and kidneys of animal models.

In order to perform one preferred embodiment of the invention, a subject is first imaged and then the polymeric contrast agent is introduced into the subject by injecting the contrast agent intravenously. The dose of the polymeric contrast agent can be in the range of about 0.01 mmoles Gd/Kg to about 0.1 mmoles Gd/Kg. The subject is then imaged at one or more pre-selected tissue sites. The one or more pre-selected tissue sites are chosen preferably is homogenous tissue free of fatty deposits, since fatty deposits tend to give high background signals. The imaged tissue site is also preferably tissue that is not normally "leaky" and therefore normally gives small signals with polymeric contrast agents. Thus, for example, kidney tissue, which performs glamelular filtration or liver which may present complications due to hepatocyte interactions with the agent are less preferred sites for imaging. In addition, the site chosen for imaging should have a reasonable degree off vascularizaton. In view of the foregoing criteria, muscle tissue is a preferred site for imaging. Normal muscle tissue gives a relatively low signal which is flat over a period of approximately one hour and which disappears after many hours due to elimination of the agent from the blood stream.

The subject is imaged, preferably beginning immediately after injection and at certain timed intervals. Preferably, the timed intervals are shortly after injection (within 10 minutes) and up to 1 hour post injection. FIG. 5, as described below, illustrates a preferred MRI imaging procedure. To determine changes in blood volume, imaging should take place within 10 minutes of contrast agent injection.

FIG. 5 shows the major components of a preferred MRI system which can be used in practicing the invention. Operation of the system is controlled from an operator console 100 which includes a keyboard and control panel 102 and a display 104. Console 100 communicates through a link 116 with a separate computer system 107 that enables an operator to control the production and display of images on the screen of display 104. Computer system 107 includes a number of modules which communicate with each other through a backplane 120. These include an image processor module 106, a central processing unit (CPU) module 108 and a memory module 113, known in the art as a frame buffer for storing image data arrays. Computer system 107 is linked to a disk storage 111 and a tape drive 112 for storage

of image data and programs, and communicates with a separate system control 122 through a high speed serial link 115.

System control 122 includes a set of modules connected together by a backplane 118. These include a CPU module 119 and a pulse generator module 121 which is coupled to operator console 100 through a serial link 125. Through link 125, system control 122 receives commands from the operator which determine the scan sequence that is to be performed.

Pulse generator module 121 operates the system components to carry out the desired scan sequence, and produces data which determine the timing, strength and shape of the RF pulses to be produced, and the timing and length of the data acquisition window. Pulse generator module 121 is coupled to a set of gradient amplifiers 127, to determine the timing and shape of the gradient pulses to be produced during the scan. Pulse generator module 121 also receives patient data from a physiological acquisition controller 129 that receives signals from a number of different sensors attached to the patient, such as electrocardiogram (ECG) signals from electrodes or respiratory signals from a bellows. Pulse generator module 121 is also coupled to a scan room interface circuit 133 which receives signals from various sensors associated with the condition of the patient and the magnet system. Through scan room interface circuit 133, a patient positioning system 134 receives commands to move the patient to the desired position for the scan.

Gradient amplifier system 127 that receives gradient waveforms from pulse generator module 121 is comprised of G_x, G_y and G_z amplifiers. Each gradient amplifier excites a corresponding gradient coil in an assembly 139 to produce the magnetic field gradients used for position encoding acquired signals. Gradient coil assembly 139 forms part of a magnet assembly 141 which includes a polarizing magnet 140 and a whole-body RF coil 152. A transceiver module 150 in system control 122 produces pulses which are amplified by an RF amplifier 151 and coupled to RF coil 152 by a transmit/receive switch 154. The resulting signals radiated by the excited nuclei in the patient may be sensed by the same RF coil 152 and coupled through transmit/receive switch 154 to a preamplifier 153. The amplified NMR signals are demodulated,

filtered, and digitized in the receiver section of the transceiver 150. Transmit/receive switch 154 is controlled by a signal from pulse generator module 121 to electrically connect RF amplifier 151 to coil 152 during the transmit mode and to connect preamplifier 153 to coil 152 during the receive mode. Transmit/receive switch 154 also enables a separate RF coil (for example, a head coil or surface coil) to be used in either the transmit or receive mode.

The NMR signals picked up by RF coil 152 are digitized by transceiver module 150 and transferred to a memory module 160 in system control 122. When the scan is completed and an entire array of data has been acquired in memory module 160, an array processor 161 operates to Fourier transform the data into an array of image data. These image data are conveyed through serial link 115 to computer system 107 where they are stored in disk storage 111. In response to commands received from operator console 100, these image data may be archived on tape drive 112, or may be further processed by image processor 106 and conveyed to operator console 100 for presentation on display 104.

The polymeric contrast agent molecules do not penetrate normal capillaries. Thus if the capillaries become leaky due to the TNF signals produced by the sepsis infection condition, the change may be detected by an increase of signal in the tissue being examined over that to be expected from blood volume effects alone in that tissue. Thus, the present methods provide clear direct signals of the quantity of interest – namely, capillary permeability.

The present polymeric contrast agents provide a benefit compared to the small molecular weight agents currently in clinical use because the small agents pass through normal capillary walls, making normal tissue appear to be leaky. Thus, signal changes due to an increase in capillary leakage are harder top detect with the small molecular weight agents currently in clinical use compared to the present polymeric contrast agents. Agents having a globular configuration are sufficiently large in hydrodynamic diameter as to remain within even diseased blood vessels and give quite small signals when probing the increased permeability of leaky blood vessels.

The present methods can be used with a number of different pulse sequences. An illustrative sequence employs a fast 3D (three dimensional) RF (radio frequency) phase spoiled gradient recalled echo pulse sequence, depicted in FIG. 6, to acquire the NMR image data. The pulse sequence "3dfgre" available on the General Electric 1.5 Tesla MR scanner sold by General Electric Company, Milwaukee, Wis., under the trademark "SIGNA" with revision level 5.5 system software is used.

As shown in FIG. 6, an RF excitation pulse 220 having a flip angle of from 40° to 60° is produced in the presence of a slab select gradient pulse 222 to produce transverse magnetization in the three-dimensional (3D) volume of interest as taught in Edelstein et al. U.S. Pat. No. 4,431,968 assigned to the instant assignee. This is followed by a slice encoding gradient pulse 224 directed along the z axis and a phase encoding gradient pulse 226 directed along the y axis. A readout gradient pulse 228 directed along the x axis follows, and a partial echo (60%) NMR signal 230 is acquired and digitized as described above. After the acquisition, rewinder gradient pulses 232 and 234 rephase the magnetization before the pulse sequence is repeated as taught in Glover et al. U.S. Pat. No. 4,665,365 assigned to the instant assignee. As is well known in the art, the pulse sequence is repeated and the respective slice and phase encoding gradient pulses 224 and 226 are stepped through a series of values to sample the 3D k-space.

The acquired 3D k-space data set is Fourier transformed along all three axes and a magnitude image is produced in which the brightness of each image pixel indicates the NMR signal strength from each corresponding voxel in the 3D volume of interest.

An initial signal is then compared with the signal enhancement observed at selected times, preferably a short time after injection (within 10 minutes) and then at several time points up to 60 minutes post injection. For highest sensitivity to measure capillary permeability, a subsequent image at about 24 hours may also be taken. The initial image after injection (within 10 minutes) provides a measure of blood volume or microvascular density, for each pixel of the image. Subsequent images then establish the rate of leakage into the tissue surrounding the capilllaries, again on a pixel by pixel basis. Maps of blood volume and of capillary permeability may then be

generated and displayed as an image or overlaid on the MR image directly. Both anatomical and physiological features will then be displayed simultaneously, giving radiologists not only the level of capillary permeability as an average quantity but also its activity as a function of position--a very desirable feature for staging and prognosis.

While specific embodiments of the invention have been illustrated and described herein, it is realized that modifications and changes will occur to those skilled in the art. It is therefore to be understood that the appended claims are intended to cover all such modifications and changes as fall within the true spirit and scope of the invention.

WHAT WE CLAIM IS:

1. A method for assessing the permeability of a blood vessel, the method comprising:

intravenously administering a polymeric contrast agent to a subject, the polymeric contrast agent having a length that is 5 to 500 times its average diameter;

obtaining at least two images of tissue of the subject; and

comparing the images to assess the permeability of a blood vessel, wherein a localized image enhancement indicates an area of increased permeability of a blood vessel.

2. A method for diagnosing sepsis, the method comprising:

intravenously administering a polymeric contrast agent to a subject having or suspected of having sepsis, the polymeric contrast agent having a length that is 5 to 500 times its average diameter;

obtaining at least two images of tissue of the subject; and

comparing the images to determine the presence of sepsis, wherein a localized image enhancement indicates the presence of sepsis.

3. A method for assessing the permeability of a blood vessel, the method comprising:

providing a polymeric contrast agent by reacting a substantially mono-activated steric hindrance molecule with a polymer, to provide a polymer-steric hindrance molecule copolymer having an elongated structure and having a degree of conjugation of 90% or greater and loading the polymer-steric hindrance molecule copolymer with an image producing entity;

intravenously administering the polymeric contrast agent to a subject

obtaining at least two images of tissue of the subject; and

comparing the images to assess the permeability of a blood vessel, wherein a localized image enhancement indicates an area of increased permeability of a blood vessel.

4. A method as in claims 1 or 2 wherein the step of intravenously administering a polymeric contrast agent comprises administering a contrast agent having a polypeptide backbone.

5. A method as in claims 1 or 2 wherein the step of intravenously administering a polymeric contrast agent comprises administering a contrast agent having a backbone formed from a polypeptide selected from the group consisting of polylysine, polyglutamic acid, polyaspartic acid and copolymers of lysine and either glutamic acid or aspartic acid.

6. A method as in claims 1 or 2 wherein the step of intravenously administering a polymeric contrast agent comprises administering a contrast agent having a polymer backbone having covalently bound thereto at least one member from the group consisting of diethylenetriaminepentaacetic acid (DTPA), 1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), 1,4,7,10-Tetraazacyclododecane-1,4,7,10- tetrakis(2-propionic acid) (DOTMA), 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid (TETA), 1,4,7,10-tetraazacyclododecane -1,4,7,10-tetrakis(3-(4-carboxyl)-butanoic acid), 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrakis(acetic acid-methyl amide), 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrakis(methylene phosphonic acid), and p-isothiocyanatobenzyl-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (p-SCN-Bz-DOTA), bis(thiosemicarbazone), derivatives of bis(thiosemicarbazone), porphyrins, derivatives of porphyrins, 2,3-bis(2-thioacetamido)propionates, derivatives of 2,3-bis(2-thioacetamido)propionates, *N,N'*-bis(mercaptoacetyl)-2,3-diaminopropanoate, bis(aminoethanethiol) and derivatives of bis(aminoethanethiol).

7. A method as in claims 1, 2, or 3 wherein the step of intravenously administering a polymeric contrast agent comprises administering a contrast agent at a dose in the range of about 0.01 mmoles Gd/Kg to about 0.1 mmoles Gd/Kg.

8. A method as in claims 1, 2, or 3 wherein the step of obtaining at least two images of tissue of the subject comprises obtaining images of muscle tissue.

9. A method as in claims 1 or 2 wherein the step of intravenously administering a polymeric contrast agent comprises administering a contrast agent having a polypeptide backbone having a length of 80 to 1500 amino acid residues.

10. A method as in claims 1, 2 or 3 wherein the step of intravenously administering a polymeric contrast agent comprises administering a contrast agent having a diameter in the range of 20 Angstroms to 50 Angstroms.

11. A method as in claims 1 or 2 wherein the step of obtaining at least two images of tissue of the subject comprises obtaining the at least two images within one hour of administration of the administration of the polymeric contrast agents.

12. A method as in claims 1 or 2 wherein the step of intravenously administering a polymeric contrast agent comprises administering a contrast agent having one or more paramagnetic entities chelated to a polymeric backbone.

13. A method as in claims 1 or 2 wherein the step of intravenously administering a polymeric contrast agent comprises administering a contrast agent having one or more gadolinium ions chelated to a polymeric backbone.

14. A method as in claim 3 wherein the step of providing a polymeric contrast agent comprises reacting a substantially mono-activated diethylenetriamine pentaacetic acid with a polypeptide.

15. A method as in claim 3 wherein the step of loading the polymer-steric hindrance molecule copolymer with an image producing entity comprises contacting the polymer-steric hindrance molecule copolymer with a solution containing gadolinium ions.

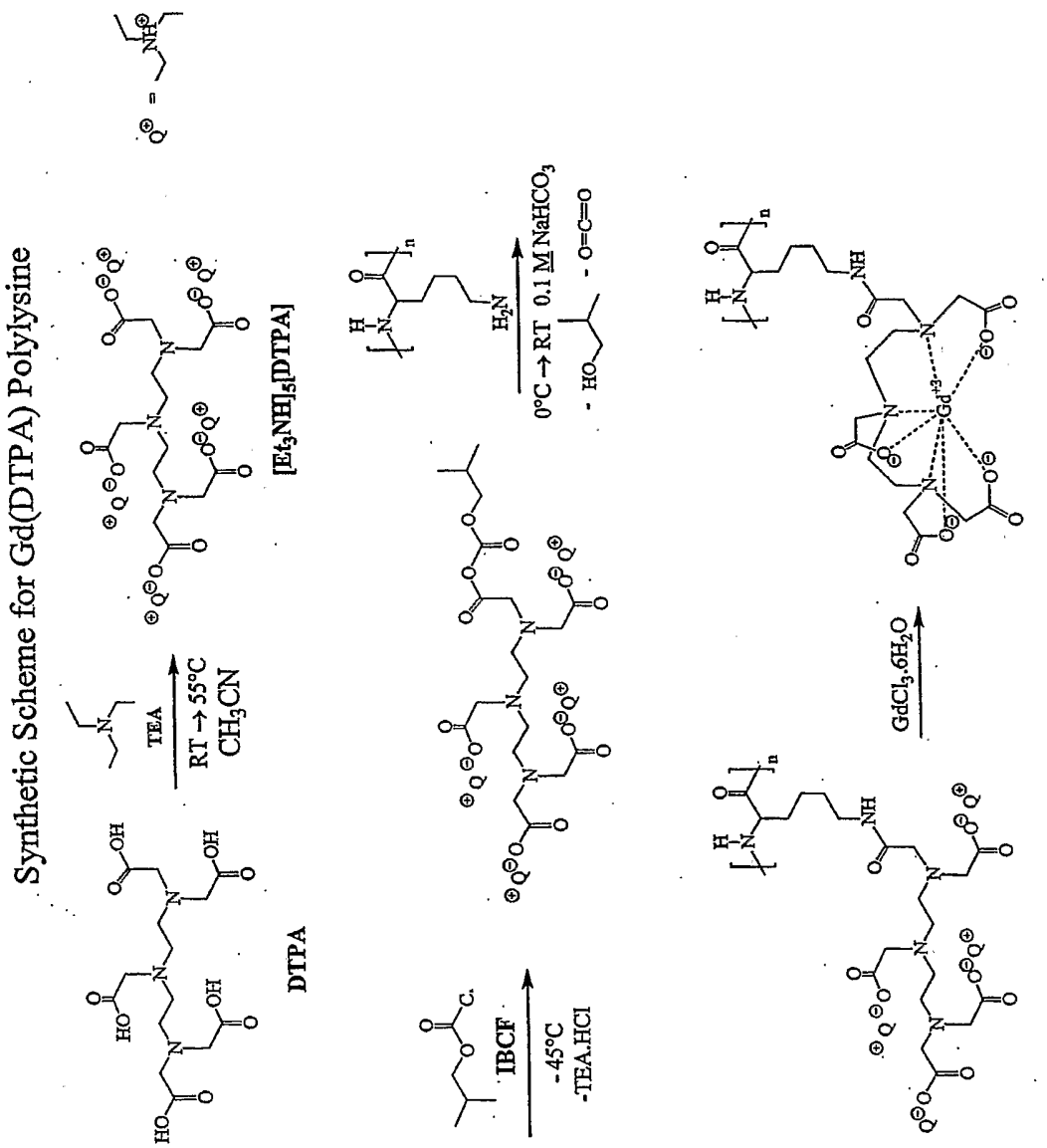

Synthetic Scheme for Gd(DTPA) Polylysine
DTPA
TEA
RT → 55°C
CH_3CN
$[Et_3NH]_5[DTPA]$
IBCF
-45°C
-TEA.HCl
H_2N
0°C → RT 0.1 M $NaHCO_3$
- O=C=O
- HO
$GdCl_3.6H_2O$

Fig. 1

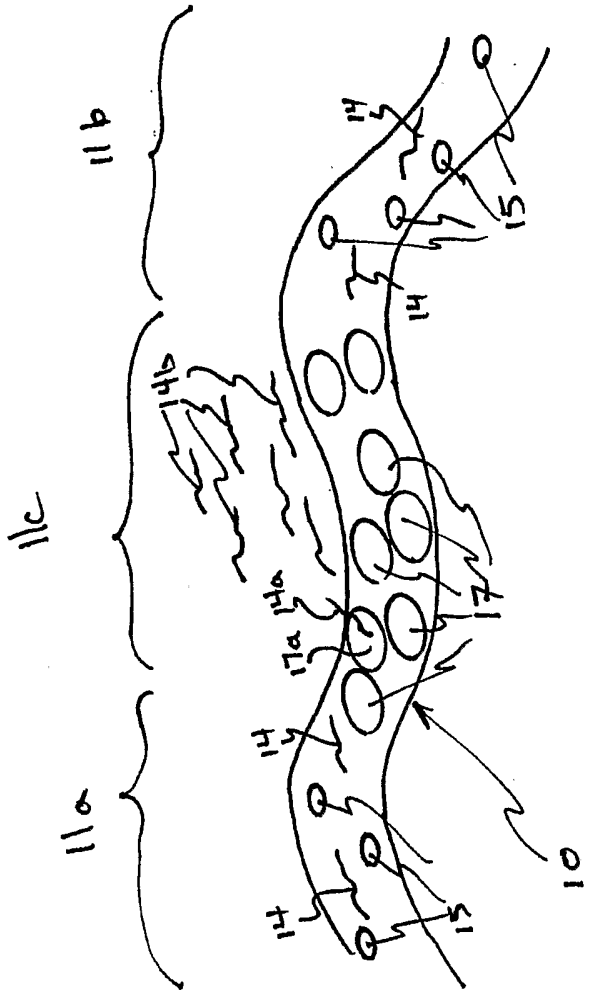


FIG. 2

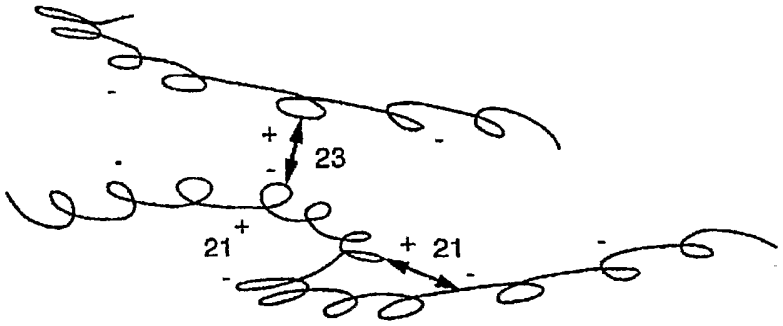


FIG. 3

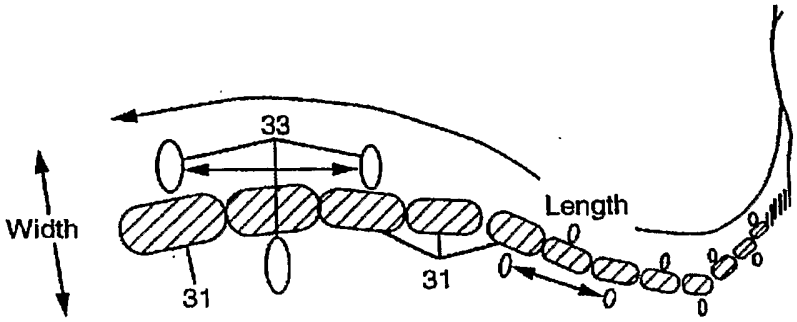


FIG. 4

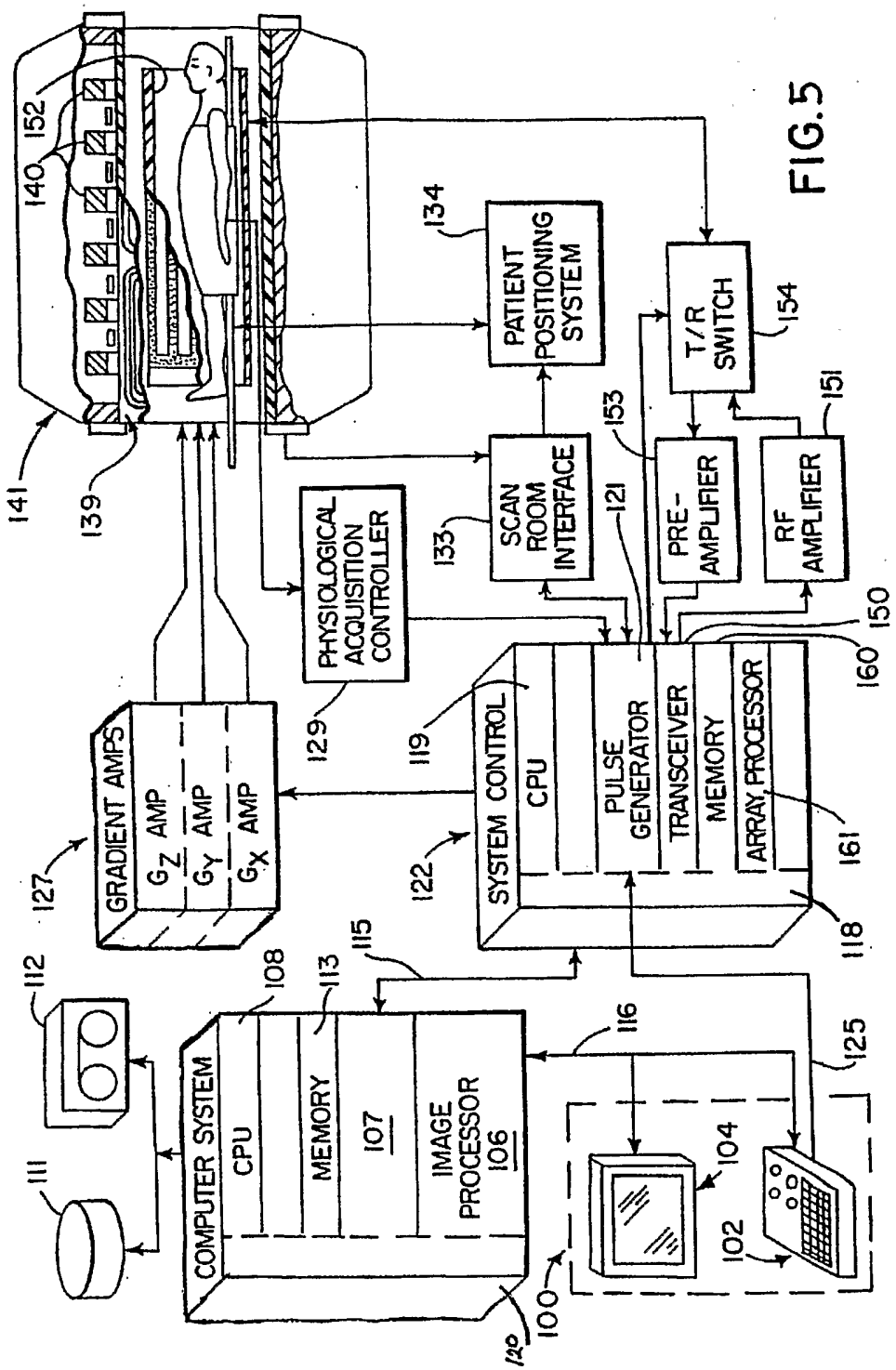
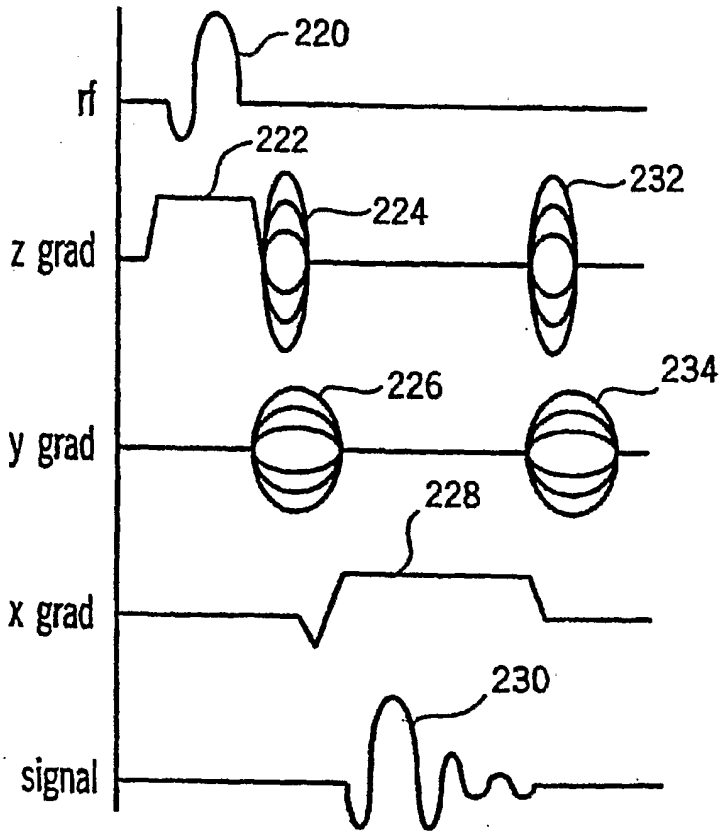

152
140
141
139
134
PATIENT POSITIONING SYSTEM
T/R SWITCH
154
153
151
SCAN ROOM INTERFACE
133
121
PRE-AMPLIFIER
RF AMPLIFIER
PHYSIOLOGICAL ACQUISITION CONTROLLER
129
150
160
GRADIENT AMPS
G_Z AMP
G_Y AMP
G_X AMP
127
119
SYSTEM CONTROL
CPU
PULSE GENERATOR
TRANSCEIVER
MEMORY
ARRAY PROCESSOR
122
161
118
115
112
108
113
116
125
COMPUTER SYSTEM
CPU
MEMORY
107
IMAGE PROCESSOR 106
104
111
102
100
120

FIG.5

FIG. 6



INTERNATIONAL SEARCH REPORT

Internati pplication No
PCT/US 03/20957

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K49/08 A61K49/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A61B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
CHEM ABS Data, BIOSIS, EMBASE, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6 235 264 B1 (UZGIRIS EGIDIJUS EDWARD) 22 May 2001 (2001-05-22) abstract; claims; figure 1 ---	1-15
X	WO 92 07259 A (BIOMED FRONTIERS INC) 30 April 1992 (1992-04-30) claims 1,17 ---	1,3
X	SCHNEIDER G ET AL: "In vivo microscopic evaluation of the microvascular behavior of FITC-labeled macromolecular MR contrast agents in the hamster skinfold chamber." INVESTIGATIVE RADIOLOGY. SEP 2000, vol. 35, no. 9, September 2000 (2000-09), pages 564-570, XP000802449 ISSN: 0020-9996 abstract --- -/--	1,3-15

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 November 2003	02/12/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Berte, M

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 03/20957

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	BOGDANOV A A ET AL: "A NEW MARCOMOLECULE AS A CONTRAST AGENT FOR MR ANGIOGRAPHY: PREPARATION, PROPERTIES, AND ANIMAL STUDIES" RADIOLOGY, OAK BROOK,IL, US, vol. 187, 1 June 1993 (1993-06-01), pages 701-706, XP000606166 ISSN: 0033-8419 abstract page 705, column 3, paragraph 3 ---	1,3-15
X	LU ZHENG-RONG ET AL: "Poly(l-glutamic acid) Gd(III)-DOTA conjugate with a degradable spacer for magnetic resonance imaging." BIOCONJUGATE CHEMISTRY. 2003 JUL-AUG, vol. 14, no. 4, July 2003 (2003-07), pages 715-719, XP002261711 ISSN: 1043-1802 abstract page 717, column 2, paragraph 2 ---	1-15
X	AREF MICHAEL ET AL: "Identifying tumor vascular permeability heterogeneity with magnetic resonance imaging contrast agents." INVESTIGATIVE RADIOLOGY. APR 2002, vol. 37, no. 4, April 2002 (2002-04), pages 178-192, XP000802465 ISSN: 0020-9996 abstract ---	1-15
P,X	US 6 537 521 B2 (UZGIRIS EGIDIJUS EDWARD) 25 March 2003 (2003-03-25) abstract; claims -----	1-15

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 03/20957

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1-15 are directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internatic pplication No
PCT/US 03/20957

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 6235264 B1	22-05-2001	US 2001028877 A1	11-10-2001
		US 2001028876 A1	11-10-2001
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		CA 2094028 A1	17-04-1992
		EP 0553273 A1	04-08-1993
		JP 6502177 T	10-03-1994
		WO 9207259 A1	30-04-1992
		US 5268165 A	07-12-1993
US 6537521 B2	11-10-2001	US 6235264 B1	22-05-2001
		US 2001028877 A1	11-10-2001
		US 2001028876 A1	11-10-2001